

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings of claims in the application:

Listing of Claims:

1-11. (Cancelled)

12. (Previously presented) A glycoPEGylated EPO peptide comprising an EPO peptide and at least one glycan and at least one poly(ethylene glycol) molecule covalently attached to said glycan, wherein said glycoPEGylated EPO peptide is made by a method comprising: adding said poly(ethylene glycol) molecule to said EPO peptide using a glycosyltransferase.

13. (Original) The glycoPEGylated EPO peptide of claim 12, comprising at least one mono-antennary glycan.

14. (Previously presented) The glycoPEGylated EPO peptide of claim 12, wherein each of said at least one glycan is N-linked and mono-antennary.

15. (Previously presented) The glycoPEGylated EPO peptide of claim 12, wherein each of said at least one glycan is N-linked and at least one of said glycans comprise said poly(ethylene glycol).

16. (Original) The glycoPEGylated EPO peptide of claim 15, wherein more than one of said glycans comprises said poly(ethylene glycol).

17. (Previously presented) The glycoPEGylated EPO peptide of claim 12, wherein each of said at least one glycan is N-linked and all of said glycans comprise said poly(ethylene glycol).

18. (Original) The glycoPEGylated EPO peptide of claim 12, comprising at least three mono-antennary glycans having said poly(ethylene glycol) covalently attached thereto.

19-22. (Cancelled)

23. (Original) The glycoPEGylated EPO peptide of claim 12 wherein said poly(ethylene glycol) is linked to at least one sugar moiety selected from the group consisting of fucose (Fuc), N-acetylglucosamine (GlcNAc), galactose (Gal) and a sialic acid (SA).

24. (Original) The glycoPEGylated EPO peptide of claim 23, wherein said sialic acid is N-acetylneuraminic acid.

25. (Original) The glycoPEGylated EPO peptide of claim 12, wherein said EPO peptide does not comprise an O-linked glycan.

26. (Original) The glycoPEGylated EPO peptide of claim 12 wherein said EPO peptide comprises at least one O-linked glycan.

27. (Previously presented) The glycoPEGylated EPO peptide of claim 26, wherein said O-linked glycan comprises said poly(ethylene glycol) covalently attached thereto.

28. (Original) The glycoPEGylated EPO peptide of claim 27, wherein said EPO peptide is recombinantly expressed in a cell.

29. (Original) The glycoPEGylated EPO peptide of claim 28, wherein said cell is selected from the group consisting of an insect cell, a fungal cell and a mammalian cell.

30. (Cancelled)

31. (Original) The glycoPEGylated EPO peptide of claim 29, wherein said cell is an insect cell.

32. (Original) The glycoPEGylated EPO peptide of claim 29, wherein said cell is a yeast cell.

33. (Original) The glycoPEGylated EPO peptide of claim 29, wherein said cell is a mammalian cell.

34. (Original) The glycoPEGylated EPO peptide of claim 33, wherein said mammalian cell is a CHO cell.

35. (Original) The glycoPEGylated EPO peptide of claim 12, wherein said poly(ethylene glycol) has a molecular weight selected from the group consisting of about 1 kDa, 2 kDa, 5 kDa, 10 kDa, 20 kDa, 30 kDa and 40 kDa.

36. (Original) The glycoPEGylated EPO peptide of claim 35, wherein said poly(ethylene glycol) has a molecular weight of 20 kDa.

37. (Original) The glycoPEGylated EPO peptide of claim 12, wherein said EPO peptide is selected from the group consisting of a naturally occurring EPO peptide and a mutated EPO peptide.

38. (Original) The glycoPEGylated EPO peptide of claim 37, wherein said mutated EPO peptide comprises the amino acid sequence of SEQ ID NO:73 having at least one mutation selected from the group consisting of Arg¹³⁹ to Ala¹³⁹, Arg¹⁴³ to Ala¹⁴³ and Lys¹⁵⁴ to Ala¹⁵⁴.

39. (Withdrawn) A method of making a glycoPEGylated EPO peptide, said method comprising the step of:

(a) contacting an EPO peptide with a mixture comprising a nucleotide sugar covalently linked to poly(ethylene glycol) and a glycosyltransferase under conditions sufficient to transfer said poly(ethylene glycol) to said EPO peptide.

40. (Withdrawn) The method of claim 39, wherein the sugar of said nucleotide sugar is selected from the group consisting of fucose (Fuc), N-acetylglucosamine (GlcNAc), galactose (Gal) and a sialic acid (SA).

41. (Withdrawn) The method of claim 40, wherein said sialic acid is N-acetylneuraminic acid (NAN).

42. (Withdrawn) The method of claim 39, wherein said poly(ethylene glycol) has a molecular weight selected from the group consisting of about 1 kDa, 2 kDa, 5 kDa, 10 kDa, 20 kDa, 30 kDa and 40 kDa.

43. (Withdrawn) The method of claim 42, wherein said poly(ethylene glycol) has a molecular weight of 20 kDa.

44. (Withdrawn) The method of claim 39, wherein said EPO peptide is recombinantly expressed in a cell.

45. (Withdrawn) The method of claim 44, wherein said cell is selected from the group consisting of an insect cell, a fungal cell and a mammalian cell.

46. (Withdrawn) The method of claim 45, wherein said cell is an insect cell.

47. (Withdrawn) The method of claim 45, wherein said cell is a yeast cell.

48. (Withdrawn) The method of claim 45, wherein said cell is a mammalian cell.

49. (Withdrawn) The method of claim 48, wherein said mammalian cell is a CHO cell.

50. (Withdrawn) The method of claim 39, wherein said EPO peptide is selected from the group consisting of a naturally occurring EPO peptide and a mutated EPO peptide.

51. (Withdrawn) The method of claim 50, wherein said mature EPO peptide has the sequence of SEQ ID NO:73.

52. (Withdrawn) The method of claim 50, wherein said mutated EPO peptide comprises the amino acid sequence of SEQ ID NO: 73 having at least one mutation selected from the group consisting of Arg¹³⁹ to Ala¹³⁹, Arg¹⁴³ to Ala¹⁴³ and Lys¹⁵⁴ to Ala¹⁵⁴.

53. (Withdrawn) The method of claim 39, wherein before step (a):
(b) contacting said EPO peptide with a mixture comprising a nucleotide-N-acetylglucosamine (GlcNAc) molecule and an N-acetylglucosamine transferase (GnT) for which the nucleotide-GlcNAc is a substrate under conditions sufficient to form a bond between said GlcNAc and said EPO, wherein said GnT is selected from the group consisting of GnT I, GnT II, GnT III, GnT IV, GnT V and GnT VI.

54. (Withdrawn) The method of claim 53, wherein said mixture comprises one GnT selected from the group consisting of GnT I, GnT II, GnT IV, GnT V and GnT VI.

55. (Withdrawn) The method of claim 54, wherein said GnT is GnT I.

56. (Withdrawn) The method of claim 54, wherein said GnT is GnT II.

57. (Withdrawn) The method of claim 39, wherein said glycoPEGylated EPO peptide comprises at least one mono-antennary glycan.

58. (Withdrawn) The method of claim 39, wherein the sugar of said nucleotide sugar is galactose and said glycosyltransferase is galactosyl transferase I (GalT I).

59. (Withdrawn) The method of claim 53, wherein before step (a) but after step (b):
(c) contacting said EPO peptide with a mixture comprising a nucleotide galactose (Gal) and galactosyl transferase I (GalT I) under conditions sufficient to transfer galactose to said EPO peptide.

60. (Withdrawn) The method of claim 39, wherein in step (a), the sugar of said nucleotide sugar is sialic acid and said glycosyltransferase is a sialyltransferase.

61. (Withdrawn) The method of claim 60, wherein said sialic acid is N-acetylneuraminic acid (NAN).

62. (Withdrawn) The method of claim 60, wherein said sialyltransferase is selected from the group consisting of $\alpha(2,3)$ sialyltransferase, $\alpha(2,6)$ sialyltransferase and (2,8)sialyltransferase.

63. (Original) A glycoPEGylated EPO peptide made by the method of claim 39.

64. (Currently amended) A glycoPEGylated EPO peptide, said EPO peptide comprising the sequence of SEQ ID NO:73 and further comprising an intact glycosyl linking group linking said EPO peptide and a PEG moiety of said glycoPEGylated EPO peptide.

65. (Original) A glycoPEGylated EPO peptide, said EPO peptide comprising the sequence of SEQ ID NO:73 and further comprising a mutation in said sequence.

66. (Withdrawn) A method of making a glycoPEGylated EPO peptide, said method comprising the steps of:

(a) contacting an EPO peptide with a mixture comprising a nucleotide sugar covalently linked to poly(ethylene glycol) and a glycosyltransferase under conditions sufficient to transfer said poly(ethylene glycol) to said EPO peptide, wherein said glycosyltransferase is a fucosyltransferase.

67. (Withdrawn) The method of claim 66, wherein said fucosyltransferase is selected from the group consisting of fucosyltransferase I, fucosyltransferase III, fucosyltransferase IV, fucosyltransferase V, fucosyltransferase VI and fucosyltransferase VII.

68. (Original) A glycoPEGylated EPO peptide made by the method of claim 66.

69. (Withdrawn) The method of claim 66, wherein said EPO peptide is expressed in a CHO cell.

70. (Withdrawn) A method of treating a mammal having anemia, said method comprising administering to said mammal an EPO peptide having one or more glycans having a glycoconjugate molecule attached to said peptide, wherein said EPO peptide is administered in an amount effective to increase the hematocrit level in said mammal.

71. (Withdrawn) The method of claim 70, wherein said mammal is a human.

72. (Withdrawn) A method of providing erythropoietin therapy to a mammal, said method comprising administering an effective amount of a glycoPEGylated EPO peptide comprising an EPO peptide and at least one glycan and at least one poly(ethylene glycol) molecule covalently attached to said glycan, wherein said poly(ethylene glycol) molecule is added to said EPO peptide using a glycosyltransferase, wherein said EPO peptide is administered in an amount effective to increase the hematocrit level in said mammal.

73. (Withdrawn) The method of claim 72, wherein said mammal is a human.

74. (Withdrawn) A method of treating a mammal having anemia, said method comprising administering to said mammal a glycoPEGylated EPO peptide comprising an EPO peptide and at least one glycan and at least one poly(ethylene glycol) molecule covalently attached to said glycan, wherein said poly(ethylene glycol) molecule is added to said EPO peptide using a glycosyltransferase, wherein said EPO peptide is administered in an amount effective to increase the hematocrit level in said mammal..

75. (Withdrawn) The method of claim 74, wherein said mammal is a human.

76. (Withdrawn) The method of claim 75, wherein said anemia is associated with chemotherapy.

77. (Withdrawn) A method of treating a kidney dialysis patient, said method comprising administering to said patient a glycoPEGylated EPO peptide comprising an EPO peptide and at least one glycan and at least one poly(ethylene glycol) molecule covalently attached to said glycan, wherein said poly(ethylene glycol) molecule is added to said EPO peptide using a glycosyltransferase, wherein said EPO peptide is administered in an amount effective to increase the hematocrit level in said patient.

78. (Previously presented) The glycoPEGylated EPO peptide of claim 12, wherein said at least one glycan is a biantennary glycan.

79. (Previously presented) The glycoPEGylated EPO peptide of claim 12, wherein said at least one glycan is a triantennary glycan.

80. (Previously presented) The glycoPEGylated EPO peptide of claim 12, wherein said at least one glycan is at least a triantennary glycan.

81. (Previously presented) The glycoPEGylated EPO peptide of claim 12, wherein said at least one glycan comprises at least two glycans selected from mono- and multiantennary glycans.

82. (Previously presented) The glycoPEGylated EPO peptide of claim 12, wherein said at least one glycan is selected from N-linked glycans and O-linked glycans.

83. (Previously presented) The glycoPEGylated EPO peptide of claim 12, wherein said at least one glycan is at least two glycans selected from N-linked and O-linked glycans.

84. (Previously presented) The glycoPEGylated EPO peptide of claim 12, wherein said EPO peptide has the sequence of SEQ ID NO:73.

85. (Currently amended) The glycoPEGylated EPO peptide of claim 12, wherein said method comprises the step of:

(a) contacting an EPO peptide with a mixture comprising a nucleotide sugar ~~covalently linked to~~ derivatized with poly(ethylene glycol) and a glycosyltransferase under conditions sufficient to transfer said poly(ethylene glycol) to said EPO peptide.

86. (Previously presented) The glycoPEGylated EPO peptide of claim 85, wherein said nucleotide sugar comprises a sugar moiety selected from the group consisting of fucose (Fuc), N-acetylglucosamine (GlcNAc), galactose (Gal) and sialic acid (SA).

87. (Previously presented) The glycoPEGylated EPO peptide of claim 86, wherein said sialic acid is N-acetylneuraminic acid (NAN).

88. (Previously presented) The glycoPEGylated EPO peptide of claim 85, wherein said method further comprises prior to step (a):

(b) contacting said EPO peptide with a mixture comprising a nucleotide-N-acetylglucosamine (GlcNAc) molecule and an N-acetylglucosamine transferase (GnT) for which the nucleotide-GlcNAc is a substrate under conditions sufficient to form a bond between said GlcNAc and said EPO, wherein said GnT is selected from the group consisting of GnT I, GnT II, GnT III, GnT IV, GnT V and GnT VI.

89. (Previously presented) The glycoPEGylated EPO peptide of claim 88, wherein said mixture comprises one GnT selected from the group consisting of GnT I, GnT II, GnT IV, GnT V and GnT VI.

90. (Previously presented) The glycoPEGylated EPO peptide of claim 89, wherein said GnT is GnT I.

91. (Previously presented) The glycoPEGylated EPO peptide of claim 89, wherein said GnT is GnT II.

92. (Previously presented) The glycoPEGylated EPO peptide of claim 85, wherein said sugar of said nucleotide sugar is galactose and said glycosyltransferase is galactosyl transferase I (GalT I).

93. (Previously presented) The glycoPEGylated EPO peptide of claim 85, further comprising prior to step (a) but after step (b):

(c) contacting said EPO peptide with a mixture comprising a nucleotide galactose (Gal) and galactosyl transferase I (GalT I) under conditions sufficient to transfer galactose to said EPO peptide.

94. (Previously presented) The glycoPEGylated EPO peptide of claim 85, wherein said nucleotide sugar of step (a) comprises sialic acid and said glycosyltransferase is a sialyltransferase.

95. (Previously presented) The glycoPEGylated EPO peptide of claim 94, wherein said sialic acid is N-acetylneuraminic acid (NAN).

96. (Previously presented) The glycoPEGylated EPO peptide of claim 94, wherein said sialyltransferase is selected from the group consisting of $\alpha(2,3)$ sialyltransferase, $\alpha(2,6)$ sialyltransferase and (2,8)sialyltransferase.